Glomalins and Their Relationship with Soil Carbon

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Abstract

The activity of bacteria and fungi is a relevant issue in the process of humification of organic matter and physical stability of the soil, standing out the role of arbuscular mycorrhizal fungi (AMF). AMF synthesize a recalcitrant glycoprotein called glomalin, with hydrophobic characteristics. GSRP (glomalin soil-related protein) is the generic product of proteins extracted from soil. The aim was to quantify GSRP and evaluate its share in the total soil organic carbon (TOC). GSRP presented a direct and positive association with soil TOC (R^2:0.73). The quantitative participation of GSRP regarding TOC (GSRP / TOC) revealed that as TOC content decreases, GSRP proportion increases. Within the TOC range explored in this paper (1.3 to 3.2%), the glomalin related protein pool of soil changes about 9%, representing between 27% and 36% of TOC. This behavior would indicate an increase of resistant carbon forms counteracting the effects of carbon loss.

Keywords: Glomalin, GSRP, argentine soils, total organic carbon

1. Introduction

In soils with low percentage of clay, structural fragility conditions are characterized by the continuous supply of carbonaceous residues and by the microbiological activity capable of transforming part of them in humic substances. The activity of bacteria and fungi is a relevant issue in the process of humification and physical stability of the soil, standing out the role of Arbuscular Mycorrhizal Fungi AMF (Gomez et al., 2007; Romaniuk et al., 2011; Romaniuk et al., 2012).

Arbuscular Mycorrhizal Fungi belong to the *Glomeromycota* phylogeny (Schussler et al., 2001), previously *Zygomycota*. These fungi present symbiotic relationships with more than eighty percent of terrestrial plants, including major commercial species such as wheat, corn, sorghum and forage species. Plant-AMF symbiosis induces physiological changes to increase photosynthetic rate, redistributing carbon and increasing root activity as a sink of carbonaceous substances in soils (Rao et al., 2012). Likewise, these soil organisms are responsible for a significant increase in the acquisition of nutrients (Smith and Read, 1997). At plant community level, it was demonstrated that AMF mediate competition between plants and co-factors determinants of plant diversity (Van der Heijden et al., 1998), and finally at the ecosystem level AMF are relevant in nutrient cycling processes and soil aggregation (Miller y Jastrow, 2000).

Arbuscular Mycorrhizal Fungi synthesize a glycoprotein called glomalin. Glomalin show recalcitrant behavior, glycoprotein nature and hydrophobic characteristics, which prevent water and nutrients losses from hyphae; therefore it is a very stable biomolecule, with a half-life in soil between 6-42 years (Wu et al., 2013).Using in vitro cultures of an AMF species, *Glomus intraradices*,, through an immunoreactive band, the amino acid chain sequence of glomalin was partially obtained; when compared with other Gen Bank accessions it resulted identical to the Hsp 60 protein with a probability value greater than eighty percent (Gadkar y Rillig 2006).

Using this sequence information, the entire gene of *G. intraradices* Hsp 60 was isolated, GiHsp 60 was designated as glomalin, with a predicted molecular weight 63.1 kDa, and an isoelectric point of 5,91. The GiHsp 60 gene is expressed strongly in extra mycelium of *G. intraradices*, indicating the mycelium is the main site for expression of glomalin genes (Purin y Rillig, 2008). The glomalin is not passively released or secreted in large quantities by growing mycelia; more than eighty percent of the glomalin produced by the AMF is strongly held and firmly embedded in the walls of the hyphae and spores, so that the main path of deposition in soil is through hyphae death and decomposition (Pellegrini et al., 2009; Driver et al., 2005).

Soil humus fractions include protein biomolecules that come from various microbial sources, but so far the isolation of glomalin was not possible. The extraction of soil – glomalin with sodium citrate and quantification through the Bradford method removes glomalin together with other protein sources (Wright et al., 2006; Araujo et al., 2015). The term GSRP (glomalin soil-related protein) is used to refer to the generic product pool of soil proteins extracted and quantified with the Bradford method, the most used methodology (Rosier et al., 2006; Whiffen et al., 2006). However, there are alternative methods to extract GSRP with bicinchoninic acid (BCA) (Stoscheck, 1990) that has shown some advantages over the traditional method of Bradford (Reyna y Wall, 2014). The GSRP were found in relative abundance (2-15 mg g^{-1}) in a wide range of soils, whether acid or calcareous (Schindler et al., 2007) and under various crops, such as vegetables, forage, cereals, and tree species. The soil GSRP presents a strong cementing ability inducing the formation of aggregates with an increased structural stability (Wu et al., 2013; Nobre et al., 2015). Besides the GSRP could act in soil remediation, sequestering toxic elements as copper (Singh, 2015). The overall objective of this work was to quantify the content of GSRP as biochemical indicator of soil quality in sandy soils with different organic carbon levels, and to assess its relationship with total soil organic carbon (TOC).

2. Materials and methods

This paper is based on a soil mensurative test performed in Carlos Casares, Buenos Aires Province of Argentina (35° 56' 06.01" S, 61° 10' 28.71" O), at the 2014/2015 season.Soils used are entic Hapludolls, loamy coarse mixed, thermic family (USDA Keys to Soil Taxonomy, 2010), and presented intensive agricultural use or different rotations with implanted pastures. The range of clay content in soil was 10 to 17.5%,. Single soil samples (n=15) were obtained under a completely random criterion, using 0-20 cm depth, within an area of 5,46 hectares, where sampling areas were selected, in order to include different degradation stages .

Reyna and Wall (2014) concluded that with the bicinchoninic acid (BCA) method, results were significantly stable compared to measurements performed by the Bradford methodology, that showed unstable results of the measured absorbance at changing time lapses or sample dilutions; so authors concluded that the BCA method has a higher precision and reproducibility compared to the Bradford method. Therefore, in this work the extraction and quantification of GSRP was conducted by the BCA (Stoscheck, 1990) method. Briefly, the extraction is performed with MM 50 sodium citrate and subjected to the action of two reagents:

Reagent A: 1 g sodium bicinchoninate (BCA), 2 g sodium carbonate, 0.16 g sodium tartrate, 0.4 g sodium hydroxide, and 0.95 gm sodium bicarbonate in 100 ml distilled water, and the pH adjusted to 11.25 with 10 M sodium hydroxide.

Reagent B: 0.4 g cupric sulfate (pentahydrate) in 10 ml distilled water.

Working reagent: 100 volumes of reagent A with 2 volumes reagent B.

A calibration curve is then performed by preparing standard solutions of bovine serum albumin (BSA); measurements show a linear trend with concentrations between 62.5 to 2500 mg / ml.

Standard process in tube: 1 ml of working reagent/ 20 µl sample. Incubation at 60 ° C for 30 minutes, then sample is exposed to room temperature and absorbance is read at 562 nm. Oxidizable carbon by Walkley and Black (Nelson y Sommers, 1982) was also carried out at soil samples; total organic carbon was calculated affecting oxidizable carbon by Richter corrected factor (Richter et al., 1973).

Statistical methods

Linear regression analysis and analysis of hyperbolic nonlinear regression model were performed with InfoStat statistical package (Di Rienzo et al., 2013).

3. Results and Discussion

The values of GSRP had a positive and significant linear behavior associated with soil total organic carbon (TOC), with p = <0, 0001 (Fig.1).

The adjustment of the linear regression model was good ($R^2 = 0.73$) which indicates the high association between both variables. Recently other authors agree with these results, as they found a significant and positive relationship between the GSRP with TOC (Nobre et al., 2015).

Regarding the quantitative participation of GSRP in TOC (GSRP/TOC), the non-linear regression analysis showed that as the total carbon in the soil decreases, the proportion of GSRP increases (Fig. 2). Within the range in TOC contents explored in this test (1.45 to 3.18% TOC) protein pool formed by the soil GSRP changes about 9%, representing between 36 and 27% of TOC. Wright and Nichols (2006), at four soils of USA, determined that GSRP homogeneously represents 13% of the TOC and that this pool is mainly located in the humin fraction. The results presented do not agree with this statement, and indicate a higher proportion of soil protein GSRP, which proved to be variable and to increase as the soil had lower carbon levels. This protein soil change between different content of total organic carbon may be linked to a differential expression of glomalin, since there is evidence that HMA abruptly increase synthesis under conditions of limited growth of the mycelium due to stressful conditions (Lovelock et al., 2004). Hammer and Rillig (2011), for example, measured glomalin expression increases in laboratory growing hyphae by increasing the NaCl concentration up to toxic levels, and by mechanical disturbances. Unfavourable conditions represented by degraded soils, low structural stability, and low carbon content, can produce increasing amounts of glomalin despite having a much lower hyphal growth (Rillig y Steinberg 2002). In soils with low clay content, there is a low formation of clay-humus complexes, and due to the lack of clay protection, carbon is easily oxidized (Parton et al., 1993). Under these conditions the proportional increase of GSRP could act as a resistant fraction or natural "buffer" decreasing loss of TOC due to increasing recalcitrant carbonaceous forms.

Under global environmental change scenarios, it was shown that GSRP increases with high concentrations of CO_2 in combination with exogenous inputs of nitrogen (Zhang et al., 2015) suggesting that the dynamic expression of GSRP is based on several factors acting together or independently. Considering an ecosystem scale and in relation to carbon sequestration capacity of soils under study, we might consider this change as a process that balances the oxidation of carbon reservoir associated with the differential expression of GSRP.

4. Conclusions

Organic carbon analysis from the perspective of biological production of GSRP allowed a better understanding of the relationship between oxidation ability, reserve aptitude and resistance to degradation. In this research the content of GSRP was associated with total organic carbon in soils with low clay content (R^2= 0.73), but their proportion increased from 27 to 36% to a decrease of TOC of 3.18 to 1.45%. This behavior would indicate an increase of resistant carbonaceous forms counteracting the effects of carbon loss.

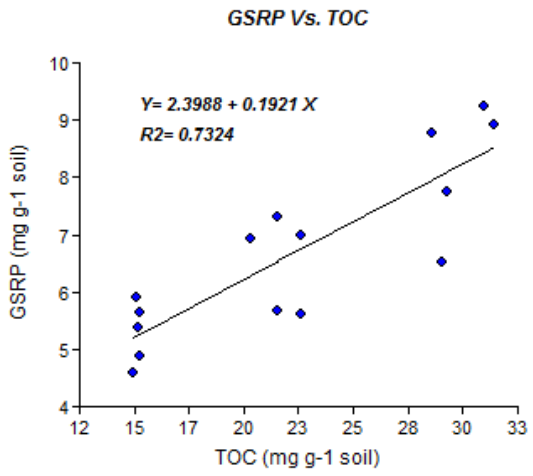

Figure 1: Linear regression of Glomalin soil-related protein (GSRP mg g^{-1} soil) and total organic carbon (TOC mg g^{-1} soil).

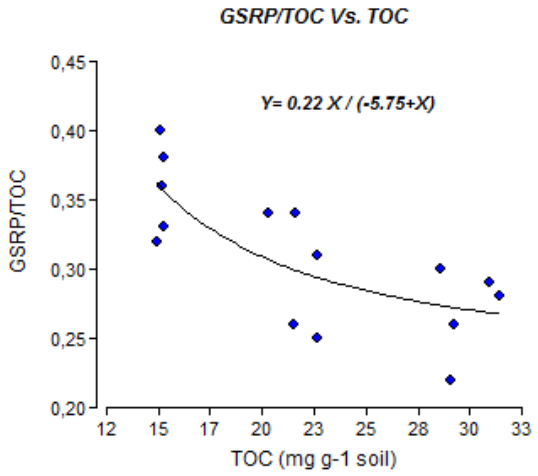

Figure 2: Nonlinear Regression between Glomalin / total organic C ratio (GSRP / TOC) and total organic carbon (TOC mg g^{-1} soil).

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